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CAN CELLULAR CEMENTUM IN BOVINE TEETH ALTER THE RESULTS OF MICROLEAKAGE TESTS?

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Abstract: Several studies use bovine teeth (DB) replacing human teeth (DH). We know that BD presents cellular cementum in the cervical region with a large concentration of cementocyte lacunae. This study evaluated whether this morphological difference has implications for microleakage results in cervical restorations. Cavity preparations (3mmx3mmx 2mm) were carried out on the buccal surface of 10 DH (3rd molar) and 10 DB (central incisor), with a cervical margin 1mm above the cementoenamel limit. Adhesive procedures (Scotchbond Multi-Purpose) were followed by the restoration with Filtek Z350XT. Polishing was carried out with aluminum oxide discs at low speed. Teeth were stored in distilled water at 37ºC/24h. The root apices were sealed and the teeth were waterproofed with cosmetic enamel, except over the restorations and 2mm below them. After drying, the teeth were immersed in 2% methylene blue at 37oC/24h. At the end, the teeth were washed/1min, and sectioned in the buccolingual direction. Microleakage was evaluated with scores of 0-4 under a stereomicroscope (40X). The statistical analysis used was Mann-Whitney (a=0,05). Kappa=1 confirmed agreement between examiners. The results showed that there was no significant difference between the groups (p=0.5205). However, it was observed that there was a difference in microleakage in the samples between the groups. In DH, microleakage always started from the tooth-restoration interface. However, in several samples in DB, microleakage began from the cellular cementum, below the cavity preparation, and continued to the pulp.

INTRODUCTION

Alternative substrates such as bovine teeth have been used to study traction tests, effects of bleaching agents, filling materials in endodontics, the adhesion resistance of orthodontic appliances, laser surface treatment, microleakage of restorative interfaces, progression of cariogenic pathogens, restorative treatment after radiotherapy and endodontics, among others. **(CURY MS 2017; PIMENTA DUTRA 2017; NOVAES, 2016, BRISO FL 2015; LIPPERT F 2015; VALERA 2015; ZAMATARO CB 2013; NAVARROS 2010; REGO FILHO F 2002)**. The difficulty of collecting human teeth for laboratory studies and the ease of collecting bovine teeth means that they can be considered substitutes for human teeth. **(SOARES 2016; YASSEN GH 2011)**. In related comparisons regarding the number of human dentinal tubules, bovine teeth did not show significant differences, and there was also consistency in relation to the diameter of the tubules. **(COSTA BM 2015; CAMARGO CH 2007).** Some concerns about applying data obtained from cattle to human teeth have been raised, as their chemistry and structure are not identical. **(YASSEN GH 2011**). The use of bovine teeth as substitutes in microinfiltration must be carefully considered, as they may present infiltration media in different ways than human teeth. **(ABUABARA A 2004).** There are morphological differences between them, the bovine tooth presents thick layers of cellular cementum in the cervical region (in the open crown, overlapping the enamel) forming a mixed stratified cellular cementum with trapped cells (cementocytes) observing in this region a non-homogeneous morphology around the tooth. **(AIMANO 1970; BOSSARDT 2008)**

In previous studies, it was observed that bovine incisors presented cellular cementum from the cementoenamel junction to the

middle third of the crown, with this layer being an extension of the cementum of the radicular cervical third, unlike the cervical cementum in human teeth, containing numerous gaps in cementocytes and fine canaliculi, which may cast doubt on the replacement of bovine teeth in research into microleakage in cervical restorations. **(BOSSARDT 1997; BOSSARDT 2008)**

The common structural description of bovine enamel also ignores much important morphological information; evidence has been found regarding the nature of the boundary zones between crystallites and prisms that has been commonly accepted until now in the enamel research community. **(YILMAZ 2018)**

Degradation of the margins of a restoration can accentuate microleakage and be associated with recurrent infiltrations, postoperative sensitivity, marginal staining, which results in shorter restoration longevity. **(SHIMAZU K 2014)** It is therefore necessary to correctly interpret the results of in-vitro tests, knowledge of the microstructural and morphological characteristics of the substrate we are working on. **(YAVUZ I 2013; ALMEIDA 2009).**

Therefore, given the above, this study aims to compare microleakage in bovine and human teeth in cervical restorations.

MATERIAL AND METHOD

This project was submitted to the Research Ethics Committee (C.E.P.) from ``*Universidade Paulista*``–UNIP, through ``*Plataforma Brasil*`` and submitted to the Research Ethics Committee on the Use of Animals – CEUA

The Research Ethics Committee, Opinion Number: 1,583,478 CAAE: 56124416.1.0000.5512.

We used 10 healthy human teeth (DH) (3rd molar), extracted for orthodontic indications, and 10 bovine teeth (DB) central incisors, obtained from a slaughterhouse. The remaining soft tissue was removed with a #15 scalpel blade and then all teeth were cleaned with a rubber cup, pumice stone and water at low speed for 15 seconds, washed in running distilled water and immersed in 0.5% chloramine until the moment of its use. The teeth obtained were distributed into 2 groups: G1=Human Tooth and G2=Bovine Tooth. A cervical cavity preparation (Class V) was performed on the buccal surface of each tooth (DH and DB) with an enamel occlusal margin and the cervical margin 1 mm above the cementoenamel limit.

The cavity preparations were made with a spherical diamond tip (KG Sorensen, 1016G, Ø= 1,6 mm and L= 19 mm) with high rotation and cooling without macro mechanical retention

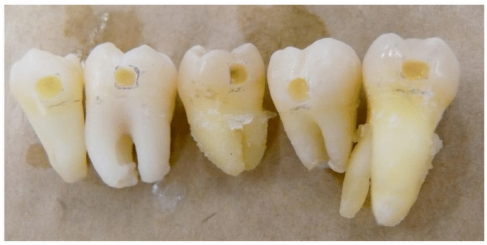

Figure 1: cavity preparations in recently extracted human teeth.

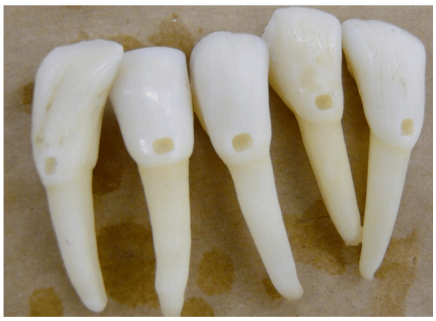

Figure 2: bovine teeth.

These preparations were standardized with the following dimensions: 3mmX3mmX2mm, measured with a digital caliper (Mitutuyo®). The diamond burs used were discarded and replaced after carrying out 5 cavity

preparations and the teeth were washed in running water before moving on to the next step. All specimens were restored with SBMP etch-rinse adhesive (Scotchbond Multi-purpose (3M ESPE, St Paul, MN, USA) and Z350 XT composite resin ((3M ESPE, St Paul, MN, USA), placed into the cavity in increments of 2 mm each and immediately light-cured at an intensity of 600 mW/cm2. Finishing was carried out with multi-laminated carbide tips, applied with light pressure and intermittently, with refrigeration and polishing was carried out with oxide discs. aluminum (Sof-Lex 3M ESPE), with decreasing grain at low speed. All materials were used according to the manufacturers' instructions. After polishing, the teeth were immersed in distilled water and left in an oven at 37o C for 24 hours. After this period, the apices were sealed with utility wax, cyanoacrylate, durepoxy and were waterproofed with nail polish, except on the restorations and 2 mm below them. After drying, the teeth were immersed in 2% blue methylene for 24 hours.

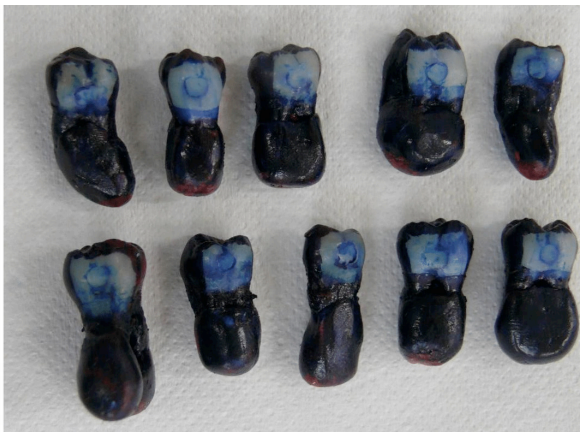

Figure 3: HUMAN teeth after polishing, the teeth were immersed in distilled water and left in an oven at 37o C for 24 hours. After this period, the apices were sealed with utility wax, cyanoacrylate, durepoxy and were waterproofed with nail polish, except on the restorations and 2 mm below them. After drying, the teeth were immersed in 2% methylene blue for 24 hours.

Figure 4: BOVINE teeth after polishing, the teeth were immersed in distilled water and left in an oven at 37o C for 24 hours. After this period, the apices were sealed with utility wax, cyanoacrylate, durepoxy and were waterproofed with nail polish, except on the restorations and 2 mm below them. After drying, the teeth were immersed in 2% methylene blue for 24 hours.

This infiltration was evaluated with the aid of a stereoscopic magnifying glass, at 40X magnification. The "rankings" for the assessment of microleakage were 0-4 (scores below). Two calibrated evaluators evaluated the margins with infiltration and classified them into an appropriate score, according to the infiltration index:

Score 0: no infiltration

Score 1: superficial infiltration of enamel only

Score 2: infiltration throughout the entire thickness of the enamel

Score 3: infiltration up to half the dentin thickness

Score 4: infiltration throughout the entire dentin thickness, up to the axial wall.

All data obtained were analyzed using the Kappa test, to verify agreement between examiners and with Mann-Whitney, adopting a=0,05, by the Bioestat program.

RESULTS

It was observed that the way in which dye penetrability occurred was different between the groups. Regarding permeability, the amount of incorporated dye/unit area was different. In DH, it always started with the tooth-restoration interface; however, in several DB samples, it started from the cellular cementum, below the preparation and continued to the pulp, probably due to the large concentration of cementocyte lacunae.

The Kappa=1 test confirmed agreement between examiners. The results showed that there was no significant difference between the groups (p=0.5205).

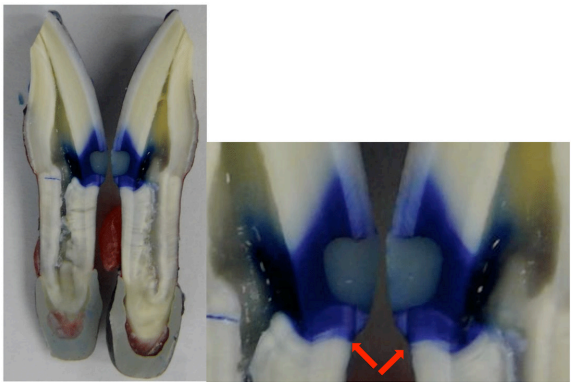

Figure 5: bovine tooth – arrow indicating infiltration by cementum

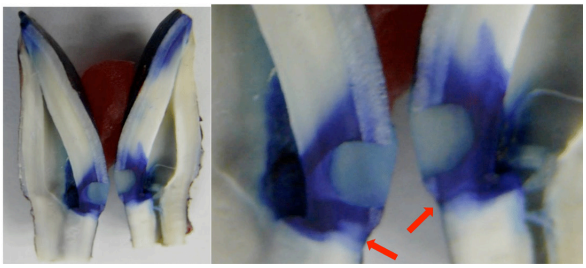

Figure 6: bovine tooth – arrow indicating infiltration by cementum

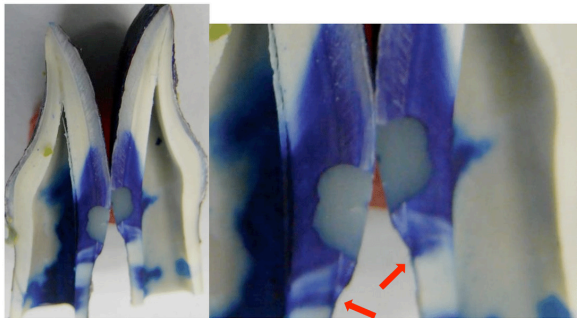

Figure 7: bovine tooth – arrow indicating infiltration by cementum

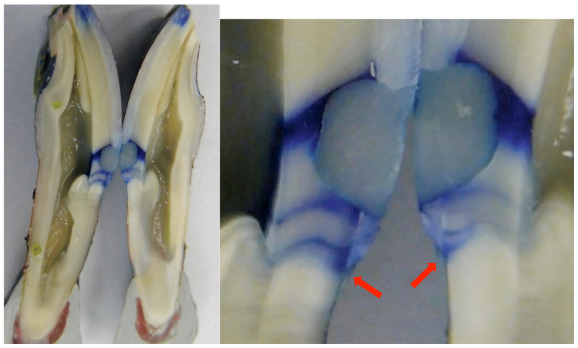

Figure 8: bovine tooth – arrow indicating infiltration by cementum

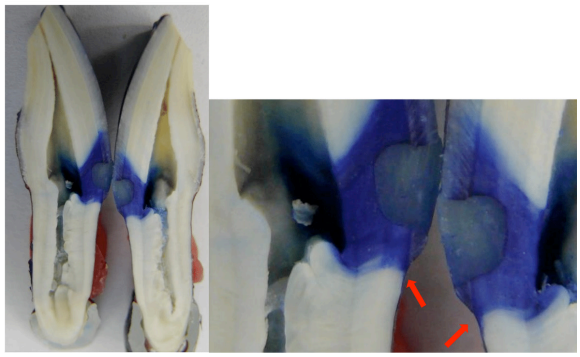

Figure 9: bovine tooth – arrow indicating infiltration by cementum

Figures 5 to 9: arrow indicating much of the infiltration as a result of the cellular cementum that covers much of the enamel in a bovine tooth below the preparation, continuing up to the pulp, probably due to the large concentration of cementocyte gaps.

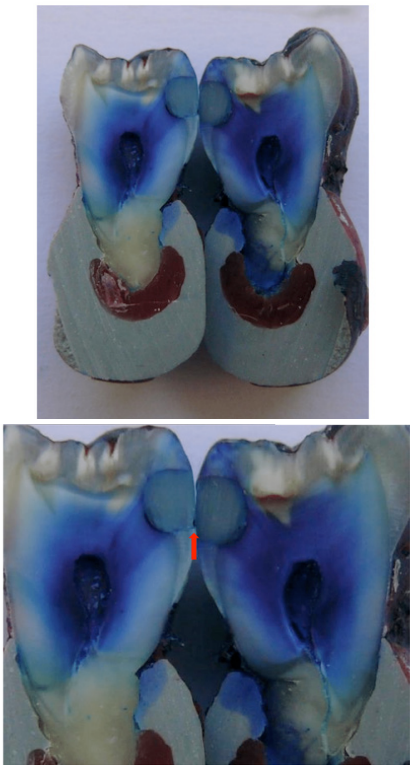

Figure 10: human tooth – infiltration through the tooth-restoration interface

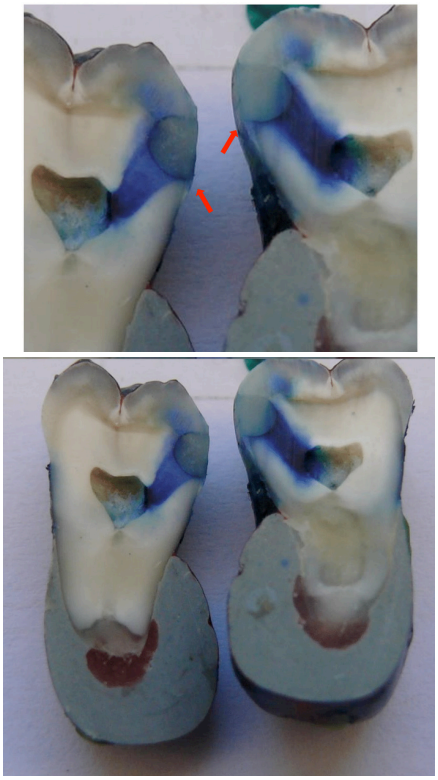

Figure 11: human tooth – infiltration through the tooth-restoration interface

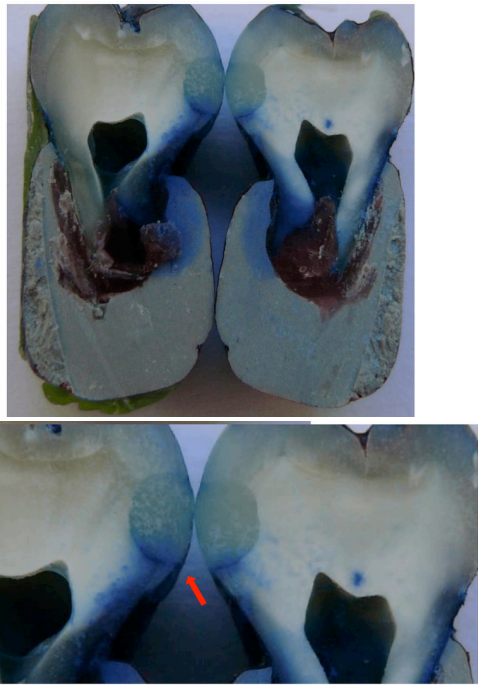

Figure 12: human tooth – infiltration through the tooth-restoration interface

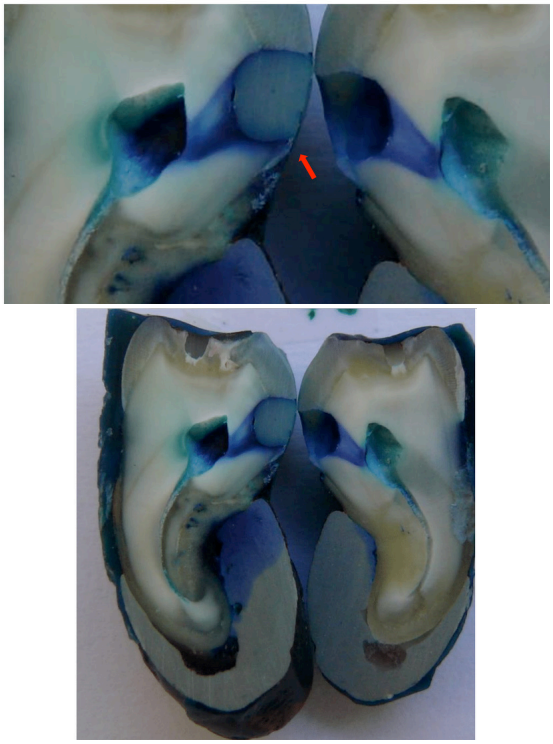

Figure 13: human tooth – infiltration through the tooth-restoration interface

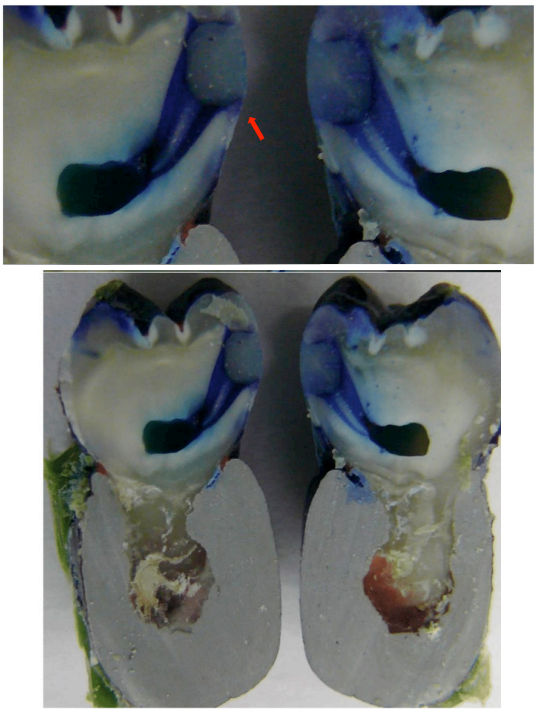

Figure 14: human tooth – infiltration through the tooth-restoration interface

Figures 10 to 14: infiltration in human teeth always started at the tooth-restoration interface.

DISCUSSION

The results of our work comparing the microleakage of bovine and human teeth showed that it may cast doubt on the replacement of bovine teeth in microleakage research in cervical restorations, since in most of the bovine substrate the microleakage started from the cellular cementum, below the cavity preparation, going to the pulp.

Validation of work on microleakage in adhesive, restorative and sealing materials must be carried out in such a way as to leave no doubt as to the sealing capacity of this material.

Bovine teeth have a structure similar to human teeth and are more easily acquired for research. Regarding research on microtraction **(CAVALLI 2018; HARUYAMA A 2016; SOARES FZM 2016),** enamel surface erosion **(MODA MD 2019; SAKAE 2018; SOARES LES 2018)**, action of chemical agents, whitening agents and surface conditioning with laser **(XAVIER RS 2017; WANG HG 2015; SAVADI OSKOEE S 2013; ZAMARATO CB 2013; NAVARRO RS 2010; REGO FILHO F 2010)**, sealing endodontic materials **(SILVA EJNL 2019)**, the work carried out confirms the use of the substrate.

Although similar, the structure of bovine enamel reveals its different shape, orientation and configuration in border regions between crystallites and prisms that are not separated by zones of prominent openings, but they are largely in contact with each other, making the type of tensions generated within the very heterogeneous bovine incisor enamel **(Yilmaz 2018)**.

The staggered arrangement of hydroxyapatite nano fibers benefits bovine enamel in terms of resistance to crack propagation, wear and fatigue **(XIAO H 2019)**. In studies on microleakage with bracket bonding, results reveal that bovine enamel can adequately replace human enamel when evaluating microleakage involving dye penetration and thermocycling. **(CAMBEK K 2013)**

The characteristics of bovine dentin are more homogeneous and the storage conditions of teeth after extraction can be better controlled. **(SOARES LES 2014).** Bovine dentin can be considered a good substitute for human dentin in adhesion studies, samples are easier to acquire, standardize and there are micromorphological similarities between the dentin forms on both substrates, showing equal amounts of superficial solid dentin. **(CASTANHO GM 2013).** It can be said that there are few differences between human and bovine dentin after adhesive treatment, however, some parameters must be evaluated, which may invalidate the bovine substrate. **(SOARES LES 2014; RETIEF DH 1990)** Microleakage in the case of cavities whose

bovine substrate margin was filled with a glass ionomer cement has been shown not to increase with artificial saliva contamination after thermocycling. **(SHIMAZU 2014)**

Bovine cementum presents morphological differentiation in relation to human cementum, with the progressive eruption of the tooth presenting an apposition of multiple layers of cellular cementum on the root surfaces and may cover the outer third of the cervical enamel, its morphogenetic characteristics and its vital function in tooth fixation do not indicate that the coronal cementum of bovine teeth corresponds to human cementum. **(AIMANO 1970)** In the bovine substrate, the cementum presents soft tissue channels and blood vessels, a characteristic similar to bones, which does not occur in human substrates, allowing the survival of cementocytes in deep layers. **(BOSSHARDT DD 2008; BOSSHARDT DD 2005)** Differences must be taken into consideration, when evaluating microleakage with bovine teeth, which is greater at the cementum margins when using this substrate. **(LOPES MB 2009).**

In our study, we used a three-phase adhesive (SBMP-3MESPE) with the aim that the generalized demineralization of enamel/dentin was completely filled with adhesive material in order to provide an improvement in adhesion with regard to the total filling of the tubules. dentin and elimination of crumbling layer, thus avoiding discontinuity of the adhesive, which could suggest that in some "gap" points there could be an exacerbated microleakage.

Work carried out with the same adhesive and the same substrates concluded that no differences were found between teeth in terms of the degree of microleakage at the enamel margin, but suggest differences at the cementum margin regardless of whether the adhesive systems tested prevent microleakage. **(ALMEIDA KGB 2009; TAVANGAR M 2016)**

Multiple factors impair the durability of resin restorations, which is why the incorporation of matrix metalloproinase inhibitors, antibacterial or remineralizing agents attempts to improve the development of adhesives and techniques to solve bonding problems, their mechanical and chemical properties. **(ZHOU W 2019; AMAIREH AI 2018; KERMANSHAHI S 2010)**

Universal, or single-phase, adhesive systems tend to simplify the work steps, making them easier to use with the promise of long-term bond stability, but degradation of the resin-dentin interface and microleakage are still the main reasons for membership failure. **(ZHOU W 2019; AMAIREH AI 2018; TAY FR 2002).**

CONCLUSION

It was conclusive that in the microleakage results there was no statistical difference between the DH, but the validation of a work on microleakage in adhesive, restorative and sealing materials must be carried out in such a way as to leave no doubts regarding the sealing capacity of this material, which was shown to us that this occurred on the restorative margin in DB.

The way in which dye penetrability occurred was different between the groups, in relation to permeability, the amount of dye incorporated/unit area, in several DB samples, started from the cellular cementum, below the preparation and continued to the pulp, probably due to the large concentration of cementocyte gaps which, compared to DH microleakage, showed that it may make the replacement of DB unfeasible in microleakage research in cervical restorations, since in most bovine substrates, microleakage started from the cellular cementum, below from the cavity preparation, going to the pulp.

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